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Original scientific paper

FERMENTED PLANT BASED PRODUCTS IN EVERYDAY NUTRITION IN MACEDONIA AND THEIR POTENTIAL OF PROBIOTIC VECTORS

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A b s t r a c t: Fermentation is one of the oldest processing techniques to extend the shelf life of perishable food and was particularly important before refrigeration. Although people can't explain the processes, mechanisms and initiators of fermentation, its peculiarity, the pleasant taste, durability of products and the positive influence over physiological and health condition of organism, encourages them to continue processing and consuming this kind of food. Fermented vegetables, peppers with cottage cheese (piperki so urda), sauerkraut (fermented cabbage), pickled cucumbers, boza (cereal fermented beverage), and apple vinegar produced in households in different regions in Macedonia can be rich source of different species lactic acid bacteria and yeasts. The aim of this study was to indicate Macedonian fermented plant products and beverages as food with positive influence over physiological and health condition and as vectors of potential probiotics.

Key words: fermented vegetables; fermented cereal beverage; health benefits; probiotic vectors

ФЕРМЕНТИРАНИ РАСТИТЕЛНИ ПРОИЗВОДИ ВО СЕКОЈДНЕВНАТА ИСХРАНА ВО МАКЕДОНИЈА И НИВНИОТ ПОТЕНЦИЈАЛ НА ПРОБИОТСКИ ВЕКТОРИ

А п с т р а к т: Една од најстарите техники на преработка на храната и продолжување на нејзиниот рок на траење, пред појавата на фрижидерите, беше ферментацијата. Иако луѓето не можеле да ги објаснат процесите, механизмите и причинителите на ферментација, сепак специфичните својства, пријатниот вкус, издржливоста на производите и позитивното влијание врз физиолошката и здравствената состојба на оние кои ја конзумираат, ги поттикнува луѓето да продолжат да прават и консумираат ваква ферментирана храна. Ферментиран зеленчук, пиперки со урда, кисела зелка (ферментирана зелка), кисели краставички, боза (ферментиран напиток од житарки) и јаболков оцет, произведени во домаќинствата во различни региони во Македонија, можат да бидат богат извор на различни видови млечно киселински бактерии и квасци. Целта на оваа студија беше да се укаже на македонските ферментирани растителни производи и пијалоци, како на храна со позитивно влијание врз физиолошката и здравствената состојба на луѓето, но и како вектори на потенцијалните пробиотици.

Клучни зборови: ферментирани растителни производи, боза, здравствени придобивки, вектори на пробиотици

INTRODUCTION

SumThe fermented food occurred very long time ago, not intentionally, but as a result of transformation of raw fruits and vegetables, when people tried to store the food during cold or drought seasons. It is a process of slow decomposition of organic substances, mainly

carb0ohydrates, into organic acids and alcohols induced by microorganisms and enzymes (FAO, 1998).

Fermented foods have heterogeneity of traditions and cultural preferences found in the different geographical areas, where they are produced. Fermentation has enabled our ancestors in temperate and cooler regions to survive during the winter season and those in the tropics to survive drought periods, using simple equipment that is readily available in most places.

Although people can't explain the processes, mechanisms and initiators of fermentation, its peculiarity, the pleasant taste, durability of products and the positive influence over physiological and health condition of organism, encourages them to continue making and consuming this kind of food. So, they actually began with the food processing with lactic acid fermenttion using indigenous natural microbiota and what is very important they passed down the family tradition to the subsequent generations. Fermented food and beverages are produced all over the world having traditional, therapeutic and economic potential. As an ancient gastronomic craft this way of presserving food is more popular in Europe, Asia, South America and Middle East. Every country even their regions have their own traditions in preparing fermented food and beverages (Baschali et al., 2017). World Health Organization (WHO) and Food and Agriculture Organization (FAO) recommended intake of a specific dose of vegetable in daily food to prevent chronic pathologies such as hypertension, coronary heart problems, and risk of strokes. Lactic acid (LA) fermentation of vegetables is a common practice to maintain and improve the nutritional and sensory features of food commodities (Demir et al., 2006; Di Cagno et al., 2013; Karovicova and Kohajdov, 2003). A great number of potential lactic acid bacteria (LAB) were isolated from various traditional naturally fermented foods (Anandharaj and Sivasankari, 2013).

In Macedonia fermented food is a part of Macedonian culture and tradition. Lactic acid fermentation is used not only for cheese making but also for plant fermentation. Vegetables and fruit fermentation is a seasonal kind of food preparation which is performed in late summer and autumn, when there is reach offer of vegetables and fruits in the markets. In the past, this was a preparation for the incoming cold winter days when fresh vegetables and fruits were unable to find. Preparation of fermented food and beverages, especially wine, in Macedonia is also a social moment when all family, friends and neighbors get together helping in grape cleaning and maceration and in the same time they socialize with each other. Today this kind of preparations for winter days are reduced since more people live in the collective housing facilities, where the preparation and storage conditions are not so appropriate and because during the whole winter season the markets are full with fresh fruits and vegetables. The markets offer various kinds of acidified vegetable (pickled cucumbers, pepperoni, mixed vegetables, peppers) that taste similar to the fermented ones.

According to the Initial list of traditional products of R. Macedonia, prepared by Slow Food Macedonia in 2019, in different regions in Macedonia, fermented vegetable is still made according to the family recipes and tradition. In this list, the simple fermented vegetable products can be found or they can be combined with some dairy products. The diversity of vegetables grown in Macedonian regions, combined with different processing conditions and technologies, cultural and religious influences provide a rich selection of tasty, quality products. In these traditional fermented products lactic acid fermentation is performed by native autochthonous microbiota that is found on the surface of the vegetables, fruits or cereals, depending on the base raw material (Ashaolu, 2019). Microorganisms (primarily lactic acid bacteria Micrococcae, Bacilli, Yeasts and filamentous fungi) play pivotal role in the process of fermentation bringing out diverse aroma components, bacteriocins, and exopolysaccharides (EPS) (Harutoshi, 2013; Leroy and De Vuyst, 2004). The epiphytic microbial population in combination with processing conditions (premises and processing tools) transforms the raw materials in products with improved characteristics such as: nutritional status, sensory appearance, expiration date, reduced toxic and anti-nutritive values (Di Cagno et al., 2013; Ashaolu, 2019). Also, when presserved properly, such food is practically harmless, with a low risk of food poisoning.

According to the International Scientific Association for Probiotics and Prebiotics (Marco et al., 2021), fermented food is defined as a food made through desired microbial growth and enzymatic conversion of food components. That means, activity of live microorganisms and their enzymes is required, because the presence and activity of enzymes only from plants, microorganisms or animals, is not enough for food to be determined as fermented. In the category of fermented food also

belongs food and beverages that pass the fermentation process but don't contain live microorganisms anymore. This is because of applied pasteurization in manner of maintaining good quality of the product (beer, wine etc.). Acidified food like pickled or a food that is chemically derived as fermented and live microorganisms have been added can't be defined as fermented because process of transformation by enzymes have not been conducted.

Plant fermented food produced in Macedonia

Shelf life of the perishable food can be improved by fermentation which is considered as the oldest technology compared to the refrigeration. Lactic acid fermentation of cabbage to produce sauerkraut has been widely studied for many years.

The Initial list of traditional products of R Macedonia, determines several fermented products prepared traditionally, using vegetable and cereal: fermented vegetables, peppers with cottage cheese (piperki so urda) and boza (cereal fermented beverage). On the menu during the winter season can also be found sauerkraut (fermented cabbage) and fermented pickled cucumber. From fermented fruits beside wine vinegar, apple or other fruit vinegar is produced in households in different regions.

Sauerkraut (fermented cabbage) is a one of the oldest traditional food product made with fermentation of fresh raw cabbage that can be processed as whole or can be chopped (Figure 1). Both ways the cabbage is cleaned first and then put in plastic or glass containers, salted with 2-4% coarse salt. After that water is poured in containers to cover the cabbage and it is left to ferment. It shuld be provide anaerobic conditions, to prevent the development of aerobic microorganisms. Therfore the thanks shuld be covered with a plastic covering. It is necessary to keep cabbage submerged in water. For the proper fermentation of cabbage the environmental conditions are very important, especially the environmental temperature. It is best if the fermentation is carried out at low temperature around 10–16˚C. On this temperature it takes approximately 30-40 days for fully fermentation and the cabbage is ready for consuming. It is usually consumed as a salad or it is cooked as different meal.

The nutritive value of cabbage used for fermentation according to Swain et al., (2014) is the following: carbohydrates 5.8%, sugars 3.2%, protein 1.28%, fat 0.1%, dietary fiber 2.5 g.

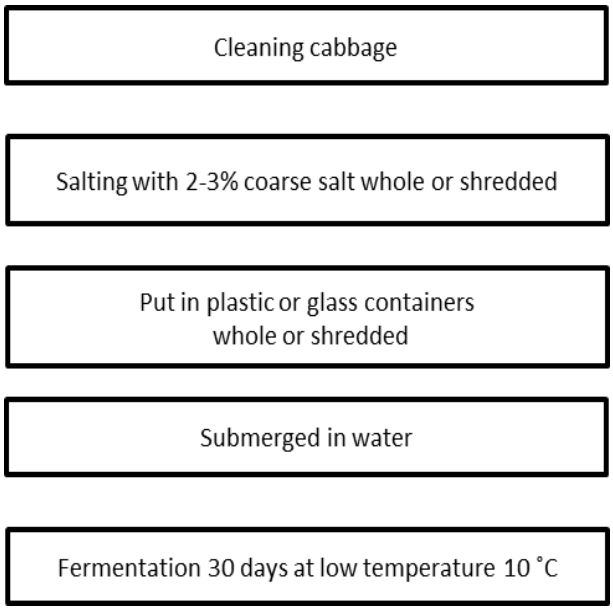


Fig. 1. Flowchart of traditional preparation of sauerkraut

Fermentation of sauerkraut usually relies on the activity of homofermentative and heterofermentative lactic-acid bacteria that are found on the raw material but also on the environment where the processing is implementing (Zabat et al., 2018). According to the (Swain et al., 2014) the main LAB which are involved in sauerkraut fermentation are: *Leuconostc mesenteroides, Lactobacillus plantarum, L. brevis* but also can be found *Weisella, Pediococcus cerevisiae* and in some cases *Enterococcus fecalis* (Zabat et al., 2018). In the beginning of the process authors emphasize the occurrence of high amount of *Pseudomonadales* from the premises and processing tools, but the restrictive conditions like salt and acidity causes changes in the conditions which favors multiplication of lactic-acid bacteria. Although the trace amount of LAB found in the first 48 hours is enough to initiate the fermentation.

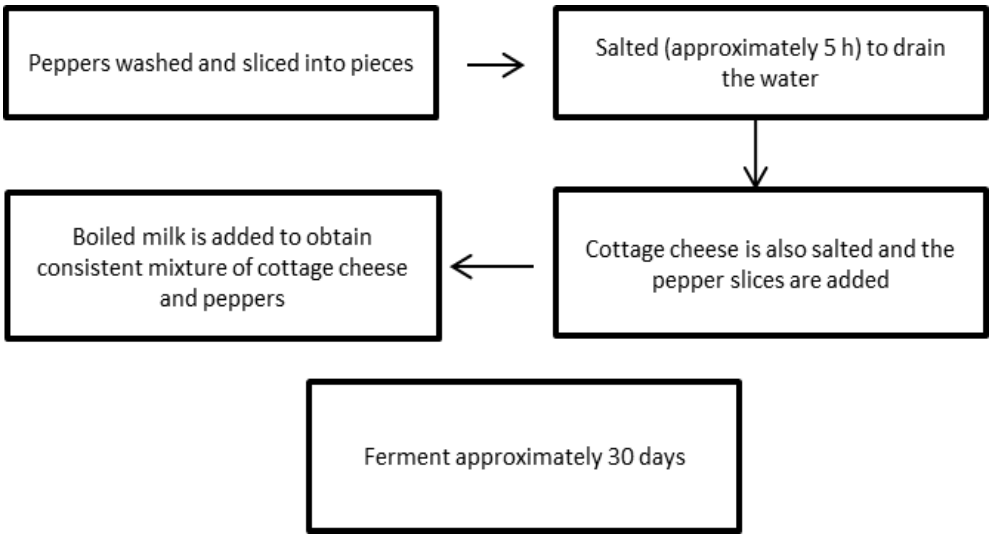
Sauerkraut brine is a byproduct of cabbage fermentation. Depend of the country where it is consumed it is called Sauerkrautsaft (Sauerkraut juice) in Germany, Moare (Ukraine, Romania), Juva (Macedonia), Rasol (Macedonia, Serbia). This beverage is very refreshing but contains a lot of salt and according to Baschali et al., (2017) contains LAB mainly *Ln. mesenteroides, Lb. brevis, Lb. sakei* and *Lb. plantarum*. According to Zabat et al. (2018) the sauerkraut juice can be further processed and used for production of carotenoids and β-glucosidase by using yeasts. Authors also suggest that consuming sauerkraut brine can contribute to healthy digestive microbiota as they may deliver probiotics to the digestive system.

A fermented vegetable is seasonal kind of food which traditionally is prepared during the autumn for the upcoming cold winter months. They are popular around the world making a great contribution to the diet. A different kind of vegetables is used as a raw material that is going to be fermented: cauliflower, green tomatoes, peppers, pickles, carrots, small melons, onion, broccoli, etc. The vegetable needs to be healthy and cleaned before it is put in a plastic or wooden container. Salt, water, acetic acid and different kinds of herbs (black pepper, dill or celery) is added. What is very important during this preparation is to allow the fermentation to be performed. This is because in most cases additives are used like: acetic, lactic, malic, and citric acid, or preservatives (sorbates, benzoates, sulphites) in order to keep vegetable not to be softened and to be crunchy during consummation. Using additives inhibits the growth of natural lactic acid bacteria and the product is not going to be fermented only acidified. Recently, fermented vegetables are rarely made because the conditions did not aloud that (high environmental temperature, lack of space for making and storage etc.). Lactic acid bacteria involved in fermentation turns raw vegetables in products with increased nutritional, sensory and shelf life properties. The adverse conditions occurring during fermentation (low pH, anaerobic condition, high salt content, low water activity) favors the growth of lactic acid bacteria over pseudomonas, enterobacteria and coryneforms, which are dominant population of microbiota in raw vegetables Di Cagno et al., (2013), Ashaolu and Reale, (2020). Traditionally fermented vegetables contain vast diversity of bacteria that may vary between respective products. The abundant lactic-acid bacteria in fermented vegetables are indigenous microbiota and depending on the type of raw vegetable are following: *Lactobacillus plantarum., Lb. pentosus, Lb. curvatus, Lb. brevis, Ln. mesenteroides ssp. mesenteroides, Weisella soli, W. cibaria, Enterococcus faecalis, E. faecium, Pediocccus pentosaceus* (Di Cagno et al, 2013). In the literature many of the above mentioned species have been described to display probiotic effects. The microbiota of fermented vegetables has a very positive influence in relation to the safety of this kind of food. LAB synthesized different kinds of organic acids, CO_2, ethanol, diacetyl, H_2O_2, antifungal components like fatty acids, phenillatyc acid, bacteriocins and antibiotics (Fan and Truelstrup Hansen, 2012). So this fermentation is a kind of bio preservation against spoilage and food poisoning microorganisms.

The nutritive value of some vegetables used for preparing fermented carrots products are given below. **Carrots** Carbohydrates 9.6%, Sugars 4.7%, Protein 0.93%, Fat 0.24%, Dietary fiber 2.8%; **cauliflower** Carbohydrates 5%, Sugars 1.9%, Protein 1.9%, Fat 0.3%,Dietary fiber 2% (Swain et al. 2014)

A pepper with cottage cheese (piperki so urda) is combined specialty eaten during winter time. It is prepared when layer of cottage cheese is put into a container and cover with layer of peppers until the container is full. Then the containers are left at low temperature (refrigerator) to ferment approximately 30 days. After that it is consumed as it is or can be used to prepare some meals like pastries or can be fried with eggs or meat. The peppers also can be sliced into pieces and salted (for approximately 5 h) to drain the water. The cottage cheese is also salted and the pepper slices are added. In order to obtain consistent mixture of cottage cheese and peppers a little boiled milk is added (1/10 of whole quantity). It is expected that the milk will improve the sensorial characteristics of cottage cheese or eliminate its strange aroma (Figure 2) (Nedelkovska and Karovska, 2019). It is expected that fermentative LAB from genus *Lactobacillus* sp. and *Leuconostoc* sp., originate from the peppers or from premises and processing tools, since cottage cheese (ricotta) is made by heating the whey obtained during cheese production, and most of lactic-acid bacteria will be inactivated or destroyed.

Pickled cucumbers are prepared using small cucumbers, salt and herbs. The cucumbers are washed, put in glass jar and poured with 3-4% brine made of water and salt. The herbs like black, pepper, dill weed can be added. In Poland garlic, blackcurrant and oak leaves are added too. Vinegar can be also added and vegetable needs to be submerged into the brine. Beside the salt, low temperature and pH, as an inhibitor factor for growing of undesirable microorganisms can be the spices that have been added. Again the main microbiota involved in the fermentation is *Lactobacillus* sp., *Leuconostoc* sp. and *Pediococcus* sp. (Ashaolu and Reale, 2020). The nutritive value of pickled cucumbers is: Carbohydrate 2.7%, Protein 0.67%, Fat 0.13%, Dietary fiber 0.8% (Swain et al., 2014).



Fig, 2. Flowchart of traditional preparation of pepper with cottage cheese (piperki so urda)

From the cereal based products **Boza** is a non-alcoholic beverage that is mostly consumed and produced in Macedonia. It is a beverage that is produced in Balkan countries, Kazachstan, Kyrgystan, Turkey. The name Boza is Turkish and has Persian origin from word buze which means millet (Petrova and Petrov, 2017). Boza can be prepared from various cereals like barley, oats, millet, maize, wheat, cheak or rice. In the past on of the boza producers was Akman in Skopje (Petrova and Petrov, 2017). Today boza is served in the confectioneries or can be commercially produced. It is characterized with sweat pleasant taste and slightly acid aroma. The color is beige to light brownish or creamy-white. The process of preparing (Figure 3) begins with cleaning and washing the grain seeds, and then milling. After that the water is added and the process of boiling starts (1–2 h). After the cooling the sugar is added and the starter culture in form of boza (1–2%) from previous preparing is used. The mixture is left to ferment 24h at the room temperature (15–25˚C). As a starter culture yogurt or sourdough can be used (Osimani at al., 2015). Boza is kept in refrigerator (4˚C) it can be bottled or can be serve in glasses or plastic cups. It is consumed during the whole year.

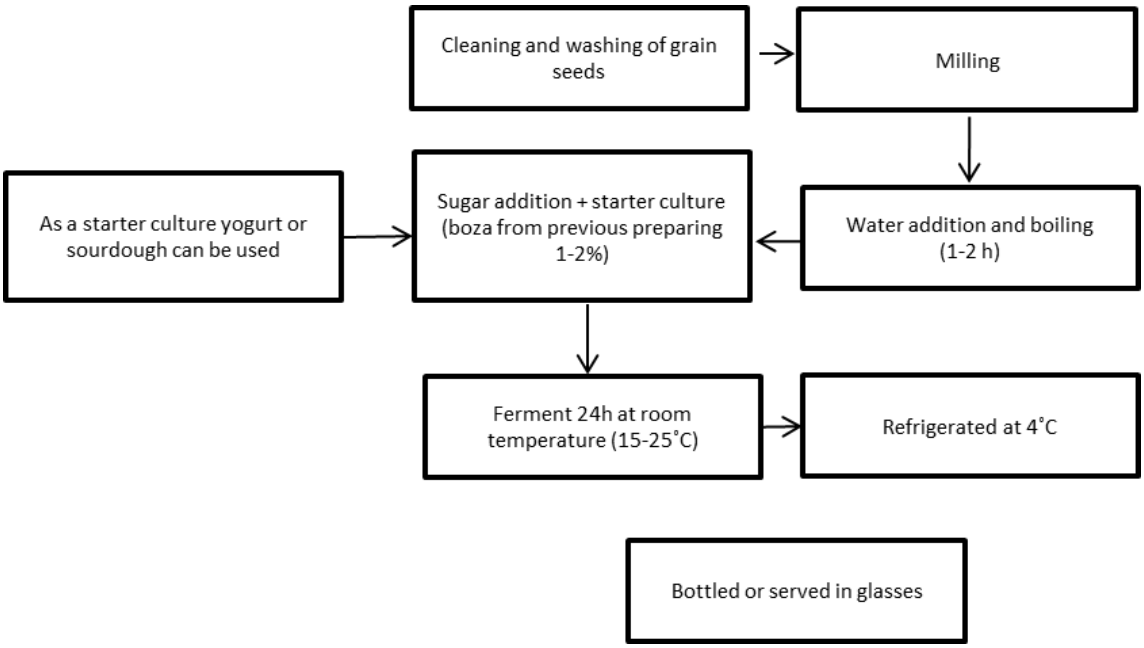


Fig. 3. Flowchart of preparation of Boza

Microflora identification of Bulgarian boza shows that it mainly consists of yeasts and lactic acid bacteria, though the lactic acid bacteria are always predominant in the microbial association with an average LAB/yeasts ratio equal to 2.4 (Gotcheva et al., 2000). The lactic acid bacteria isolated has been identified as *Lactobacillus plantarum, Lb. acidophilus, Lb. fermentum, Lb. Coprophilus, Leuconostoc raffinolactis, Ln. Mesenteroides* and *Ln. brevis*. The yeasts isolated comprise *Saccharomyces cerevisiae*, *Candida tropicalis, C. glabrata, Geotrichum penicillatum* and *G. Candidum* (Gotcheva et al., 2000). According to the Petrova and Petrov, (2017) boza contains 0.50–1.61% protein, 12.3% carbohydrate (8–12% sugar), 75–85% moisture, 0.4-0.5% lipids, 29 mg Ca, 1.3 mg Fe, 95 mg phosphorus, 1 mg Zn and vitamins niacin, riboflavin and tiamin. pH ranges from 6.7 at the beginning to 3.4-3.9 after the fermentation. Boza prepared from chickpea flour contains more protein 8.46%, more minerals like Ca (268.1 ppm), K (525.3 ppm), P (199.2 ppm), Zn (3.13 ppm), Mn (1.14 ppm), and sugar (0.05%). Oxalic, lactic, pyruvic, acetic and malic acids were also found. The content of alcohol varies from 0.02-0.79%. A similar product in Egypt contains 7% alcohol and it is consumed as a beer (LeBlank and Todorov, 2011).

Regarding microbiota content in boza the LAB and yeast are present causing lactic acid and alcoholic fermentation. The literature data indicates that LAB (70–98%) is dominant microbiota over yeasts (Gotcheva et al., 2000; Kivanc et al., 2011). The lactic acid bacteria (LAB) species found in this beverage varies depending on samples. According to the Petrova and Petrov, (2017), Osimani at al., (2015) the most frequently isolated LAB from boza are *Leuconostoc paramesenteroides, Leu. mesenteroides* subsp. *dextranicum, Lb. Fermentum, Lb. Plantarum, Lb. acidophilus Lb. fermentum Lb. paracasei* subsp. *paracasei, Lb. coryniformis* and *Lb. Fermentum, Lb. casei, Lb. buchneri* and *Lb. parabuchneri, Weissella confusa, Leu.citreum, Pediococcus parvulus, P. ethanolidurans* and *Lc. lactis.*

Yeasts were represented by following species: *Saccharomyces uvarum, S. cerevisiae, Geotrichum candidum, Candida tropicalis, C. glabrata, Torulospora spp., Pichia fermentas, P. norvegensis, Galactomyces geotricum, G. Candidum* and *G. fragrans.*

Apple vinegar is fermented fruit that is prepared in households in almost all regions in Macedonia. It is preferred to be made from variety apples which contain no preservatives. For the vinegar preparation the apples need to be healthy and washed before they are cut into pieces. The apple pieces are put into containers and sugar or honey and water is added. It is recommended the apple parts to be submerged into the water and to add less sugar if apples are sweet. The containers are left to the room temperature 20-25˚C 4-5weeks. First the alcohol fermentation is performed by indigenous yeasts using sugar which is transformed into carbon dioxide and alcohol. After that alcohol has been fermented to acetic acid under aerobic conditions thanks to the activity of acetic acid. After the fermentation is done the vinegar is filtered through canvas or cloth and the apples are squeezed to drain the liquid from them. The apple vinegar is stored in refrigerator in closed bottles and can be used for salads or meals preparation. The research of Song et al., (2019) indicates that dominant bacteria in apple vinegar are *Lactococcus, Oenococcus* and *Acetobacter, Gluconobacter, Komagataeibacter genus Saccharomycodes ludwigii, Candida ethanolica*. The dynamics of these bacteria varies during the fermentation process and their mutual activity contributes in creation of specific vinegar aroma and taste.

The Apple vinegar is not only used to enhance the food taste or for its preservation, but also for health improvement. According to Şanlier et al., (2019) vinegar consumption seems to help in reducing risk of obesity, cancer, diabetes and atherosclerosis.

Fermented food and health benefits

Positive effects connected to fermented foods have been empirically known for centuries. In many cultures, fermented foods are heritage foods and an integral part of local traditions, probably because fermentation was the only way to preserve foods (Şanlier,et al., 2019).

The use of fermented products and beverages in everyday nutrition seems to be very popular in last decades since the modern society propagates the importance of nourishment with healthy and quality food (Figure 4). On the other hand the traditional prepared food is also on demand because of it better taste and sensorial characteristics and because contains low amount of additives and preservatives (Ashaolu and Reale, 2020). Lactic acid fermentation is inexpensive and most effective way of food processing that also prolong shelf life of food. It is most used in less developed countries as a method of conserving and storage of food.

There are old written documents that witnesses using of fermented beverages in Egypt, Rome, Greece, Mesopotamia and China as pain relievers or

to prevent or treat diseases (Baschali et al., 2017). For example Koumiss was used by Russian doctors in tuberculosis and diarrhea treatment, Sorghum beer was used to prevent pellagra in South Africa and using of fermented gruels by children in Tanzania decreased the intensity of diarrhea occurrence. Till now it seems only sufficient studies from consumption of yogurt and dairy products are made to prove the benefit of fermented food consumption. According to this studies consumption of yogurt helps in reduction of adiposity factors, type 2 diabetes and cardiovascular disease. Although there are not enough research results for benefits of consuming fermented vegetables and fruits are needed, some things are well known (Marco et al., 2021).

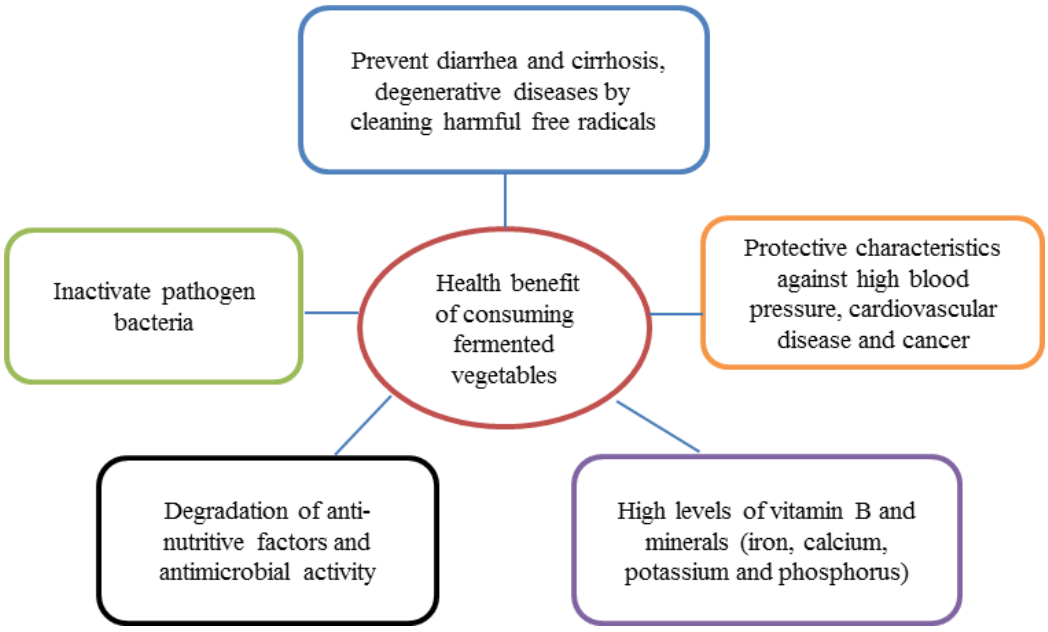


Fig. 4. Nutritional and health benefits of consuming fermented vegetables and cereals

Health benefits of fermented food are result of its microbiota activity more precisely of lactic-acid bacteria and yeasts. First of all they prevent the food spoilage and inactivate pathogen bacteria by carbohydrates oxidation to alcohol, organic acids and CO_2. The fermented vegetables and fruits contain antioxidants that can help in prevention diarrhea and cirrhosis as well as degenerative diseases by cleaning the harmful free radicals in the body (Swain et al., 2014).

The literature data (Di Cagno et al., 2013, Şanlier et al., 2017) emphasize the beneficial effect of fermented food mostly dairy products which can reduce blood cholesterol levels, increase immunity, can play important role in protection of osteoporosis, diabetes, obesity, atherosclerosis alleviating lactose intolerance symptoms. The authors indicates that the levels of B_2, B_9, B_{12} and vitamin K increase in fermented food since microbiota like LAB or yeasts are involved in their production. Also the melatonin is synthesized and gamma amino butyric acid which have some protective characteristics against high blood pressure, cardiovascular disease and cancer. These characteristics are results of bacteria activity which degrade proteins and create bioactive peptides. But the positive effects of consuming fermented vegetables, fruits and cereals should not be neglected. Fermented cabbage contains high levels of vitamin C and phenols that have antioxidant capacity, high levels of vitamin B and minerals such as iron, calcium, potassium and phosphorus (Şanlier et al., 2017). According to the Di Cagno et al., (2013), Swain et al., (2014) lactic acid bacteria isolated from raw and fermented vegetables can have functional activities that can improve human health. The strains of *Lb. fermentum* can have immune enhancing effect. The strains of *Lb. plantarum* can improve adhesion to intestine epithelial cells, can stimulate immune-mediators, lowers blood cholesterol to degrade the anti-nutritive factors and can have antimicrobial activity towards *Salmonella*, *Stapylococcus aureus* and *E. coli* (synthesis of bacteriocine, hydrogen peroxide, organic acids).

With fermentation processes the nutritive value of food can be improved. This is because some nutrients can be more available and can be used. The protein catabolism during fermentation (performed by microorganisms) provides peptides or amino acids that can be resorbed or further digested in gut, fat can be metabolized to free fatty acids that can give new sensorial characteristics to the fermented food; the concentration of some high-calories sugars (saccharose, lactose) can be reduced as a result of microorganism's activities and the glycemic index could be reduced and the food tolerability can be improved. Fermentation can cause detoxification reactions like degradation of linamarin in bitter casava, removal of the anti-nutritive factors like inactivation of trypsin inhibitor or phytic acid in cereals. Fermentation can result with improving availability of bioactive compounds like phenols and tannins (Şanlier et al., 2017, Singh et al. 2014, Swain 2014).

The consummation of cereal based fermented product like Boza can also be health beneficial because contains different kinds of minerals, vitamins, proteins and fibers. It also contains free fatty acids responsible for the specific taste which also have antibacterial activity (Şanlier et al., 2017).

The cereals used for Boza preparation contain different kinds of fibers that have bulking, viscosity and fermentation activities which influenced the function of the human organism. The bulking fibers are not or minimally fermented and can't be absorb, so their function is in regulation of intestinal activity. The viscous fibers are responsible for thickening the content of intestine that prevents sugar and fat absorption. The fermentative fibers are substrate that can be used by LAB. The fermentation process is usually done in the large intestine and they stimulate the growth lactic-acid bacteria in colon that improve host health (Petrova and Petrov, 2017). The health benefits of Boza according to the authors came from the content of β-glukan in cereals that Boza is produced from. The content of ω-3 and ω-6 fatty acids helps in preventing or decreasing the risks of coronary disease, myocardial infarction, high blood pressure, different types of cancer (breast, colon, prostate). Also this beverage contains Angiotensin I-converting enzyme that regulates blood pressure and fluid-salt balance in humans. Baschali et al., (2017) indicates that some biogenic amines like putrescine, spermidine and tyramine can be found in boza. These components might cause appearance of migraine at humans but more investigations are needed.

Fermented vegetables as probiotics vectors

The term "probiotic" refers to a preparation of defined microorganisms, or a product containing viable micro-organisms, (mainly *Lactobacilli* and *Bifidobacteria*) in sufficient numbers to alter the microflora in an intestinal compartment of the host and bring beneficial health effects (Kalliomaki et al., 2001; Schrezenmeir and de Vrese, 2001; Brown and Valiere, 2004) (Table 1).

Consumers are familiar with the fact that fermented foods contain living microorganisms. Thus, the use of probiotics as starter organisms combines the positive impact of fermentation and probiotic cultures (Heller, 2001; Saxelin et al., 2005).

Fermented food, including fermented vegetables, sometimes can be labelled as probiotic but differences have to be made. According to the consensus panel organized under the auspices of ISAPP (Marco et al., 2021), to say that some food contains probiotic several things are important to define.

– Food needs to contain live microorganisms in defined amounts during products shelf life,

– To have proof that microorganisms provide health benefit to the consumers and this to be clinically demonstrated by well-controlled study;

– To contain microorganisms which are determinate to the strain level and to be genome sequenced.

Table 1.

Beneficial effect of probiotic bacteria according to Gibson and Roberfroid (1995)

Protection of gastrointestinal and general infections with mucosal entry
Cholesterol reduction
Reduction of intestinal pH
Reduction of ammonia and other compounds
Increase of lactose tolerance and digestion
Production of B vitamins
Positive influence on intestinal microflora
Restoration of the normal intestinal microflora after antibiotic treatment
Improvement of intestinal functioning
Stimulation of the immune response

If fermented food contains live microorganisms but there is no evidence for their health benefit and they are not precisely defined (determinate to strain level and genome sequenced), can't be labelled as probiotic only as fermented food (Marco et al., 2021).

The interest of consumers for functional food and beverages which contains no lactose and has no animal origin is growing as a result of ongoing trend of vegetarianism and frequent occurrence of lactose intolerance in humans. That's why more assessments were made to prove the possibilities of fruits and vegetables to be reliable probiotic vectors (Di Cagno et al., 2013). According to the authors it is necessary for probiotics to stay in large quantity for a longer time in the carrier matrix and not to interact with the matrices nutrients because can change the products sensorial characteristics. It was proven that tomato, carrot, cabbage and artichokes juice are suitable for probiotic fermentation and the quantity of population can be in a range of 8 log cfu/ml (Rivera-Espinoza and Gallardo-Navarro, 2010).

The Macedonian fermented vegetables are carriers of autochthones microbiota but unfortunately this populations and their probiotic capacity is not fully studied. This is necessary because a new species can be found and needs to be evaluated. The potential of microbiota needs to be very well observed, clinical studies must be done to prove the probiotic capacity of microorganisms. It will be ideally if researched microbiota showed fermented and probiotic activity (strains with multifunctional capacity), which can stay longer period of time in product during fermentation and marketing and will not change its activity when entering human gut (Nuraida, 2015). If a carrier of probiotic culture is fermented vegetable it is necessary to prove that microenvironment (pH, temperature, water activity, acidity, synthesis of exopolysaccharide, etc.) of fermented vegetable is appropriate to additional microbiota, which guarantee survival and maintain of the high number of cell (10^8-10^9 cfu/g) (Singh et al., 2014). According to the authors the probiotic strains must not interact with carrier matrix because the change of taste and aroma is not acceptable for consumers. Also the protective substances that have been added during fermentation like salt or some spices, the bacteriocine or other inhibitory factors synthesized by fermentative LAB during fermentation can negatively influence probiotic survival. They also must survive gastric acid, bile salts, influence of other intestine bacteria, anaerobic condition and toxic metabolites produced during digestion and to inhabit intestinal tract. The creation of functional food by addition of probiotics is a real challenge. Further complex investigations are needed first to determinate and to classify fermenting bacteria or yeasts, than to isolate them and to investigate their technological characteristics, after that prove their health benefit by regular consumption of fermented food and to assess their probiotic activity.

In a conclusion it can be emphasized that R.N. Macedonia is reach with vegetables and fruits and their fermented version consumed during winter season since in the summer the fresh vegetables are dominant food in everyday nutrition. In summer the only fermented food that is consumed is fermented dairy products (yogurt, cheese) and vinegar. It is very important to find out what kind of lactic-acid bacteria inhabits vegetables and fruits and what are their fermentable capacities. Perhaps species with good probiotic characteristics will occur in Macedonian fermented products. Using word "probiotic" should be restricted if there is no proof for health improvement, strain determination and strain sequencing. Instead the expression fermented food can be used if it is necessary to emphasize the presence of live bacteria in fermented food.

It is also important to promote local and regional Macedonian food because is most suitable for everyday nutrition and to promote the importance of seasonal products consumption instead of imported non seasonal food.

During preparation of fermented products, vegetables especially the fermentation process is necessary. Artificial or chemical derived fermentation (acidification) will only provide acid taste of food without health benefits for consumers. Contrary the preservatives used during food preparation can have adverse effects.

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